Amendments to the Specification under Revised 37 C.F.R. § 1.121

Please amend the specification at page 2, lines 8-18 to read as follows:

The invention provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence as set forth in either SEQ ID NO: 1 or SEQ ID NO: 4;

(b) the nucleotide sequence of the DNA insert in American Type Culture Collection (ATCC) Deposit Nos. PTA-1753 or PTA-1755;

(c) a nucleotide sequence encoding the polypeptide as set forth in either SEQ ID NO: 2 or SEQ ID NO: 5;

(d) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a) - (c); and

(e) a nucleotide sequence complementary to any of (a) - (c).

Please amend the specification at page 28, lines 3-14 to read as follows:

Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are described in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux *et al.*, 1984, *Nucleic Acids Res.* 12:387; Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-10). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (Altschul *et al.*, *BLAST Manual* (NCB National Library Medicine NIH, Bethesda, MD); Altschul *et al.*, 1990, *supra*). The well-known Smith Waterman algorithm may also be used to determine identity.

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Please amend the specification at page 34, line 30 to page 35, line 22 to read as follows:

Where all or only a portion of the flanking sequence is known, it may be obtained using PCR and/or by screening a genomic library with a suitable oligonucleotide and/or flanking sequence fragment from the same or another species. Where the flanking sequence is not known, a fragment of DNA containing a flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion to produce the proper DNA fragment followed by isolation using agarose gel purification, ~~Qiagen~~® column chromatography (QIAGEN Inc., Chatsworth, CA), or other methods known to the skilled artisan. The selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

An origin of replication is typically a part of those prokaryotic expression vectors purchased commercially, and the origin aids in the amplification of the vector in a host cell. Amplification of the vector to a certain copy number can, in some cases, be important for the optimal expression of a Secs-1 polypeptide. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector. For example, the origin of replication from the plasmid pBR322 (New England Biolabs, Inc., Beverly, MA) is suitable for most gram-negative bacteria and various origins (*e.g.*, SV40, polyoma, adenovirus, vesicular stomatitus virus (VSV), or papillomaviruses such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (for example, the SV40 origin is often used only because it contains the early promoter).

Please amend the specification at page 41, lines 15-30 to read as follows:

Preferred vectors for practicing this invention are those which are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pcDNA3.1 (Invitrogen Corp., San Diego, CA), pBSII (Stratagene Corp., La Jolla, CA), pET15 (Novagen, Inc., Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech Laboratories, Inc.,

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Palo Alto, CA), pETL (BlueBacII, Invitrogen Corp.), pDSR-alpha (PCT Pub. No. WO 90/14363) and pFastBacDual (~~Gibco~~ GIBCO-BRL, Grand Island, NY).

Additional suitable vectors include, but are not limited to, cosmids, plasmids, or modified viruses, but it will be appreciated that the vector system must be compatible with the selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript® plasmid derivatives (a high copy number ColE1-based phagemid, Stratagene ~~Cloning Systems~~ Corp., La Jolla, CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products (*e.g.*, TOPO[[™]] TA Cloning® Kit, PCR2.1[[®]] plasmid derivatives, Invitrogen Corp., Carlsbad, CA), and mammalian, yeast or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech Laboratories, Inc., Palo Alto, CA).

Please amend the specification at page 43, lines 16-20 to read as follows:

Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described, for example, in Kitts *et al.*, 1993, *Biotechniques*, 14:810-17; Lucklow, 1993, *Curr. Opin. Biotechnol.* 4:564-72; and Lucklow *et al.*, 1993, *J. Virol.*, 67:4566-79. Preferred insect cells are Sf-9 and Hi5 (Invitrogen Corp.).

Please amend the specification at page 46, lines 8-20 to read as follows:

The purification of a Secs-1 polypeptide from solution can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (Secs-1 polypeptide/hexaHis) or other small peptide such as FLAG (Eastman Kodak Co., New Haven, CT) or *myc* (Invitrogen Corp., Carlsbad, CA) at either its carboxyl- or amino-terminus, it may be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag.

For example, polyhistidine binds with great affinity and specificity to nickel. Thus, an affinity column of nickel (such as the ~~Qiagen~~® nickel columns of QIAGEN Inc.) can be used for

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purification of Secs-1 polypeptide/polyHis. *See, e.g., Current Protocols in Molecular Biology* § 10.11.8 (Ausubel *et al.*, eds., Green Publishers Inc. and Wiley and Sons 1993).

Please amend the specification at page 61, lines 16-28 to read as follows:

Another *in vitro* assay that is useful for identifying a test molecule which increases or decreases the formation of a complex between a Secs-1 polypeptide binding protein and a Secs-1 polypeptide binding partner is a surface plasmon resonance detector system such as the BIAcore® assay system (Pharmacia AB Corp., Piscataway, NJ). The BIAcore® system is utilized as specified by the manufacturer. This assay essentially involves the covalent binding of either Secs-1 polypeptide or a Secs-1 polypeptide binding partner to a dextran-coated sensor chip that is located in a detector. The test compound and the other complementary protein can then be injected, either simultaneously or sequentially, into the chamber containing the sensor chip. The amount of complementary protein that binds can be assessed based on the change in molecular mass that is physically associated with the dextran-coated side of the sensor chip, with the change in molecular mass being measured by the detector system.

Please amend the specification at page 84, lines 15-19 to read as follows:

Deposits of cDNA encoding murine Secs-1 polypeptide and human Secs-1 polypeptide, subcloned into pSPORT1 (~~Gibco~~ GIBCO-BRL) and p7T73D (Pharmacia AB Corp.), and having Accession Nos. PTA-1753 and PTA-1755, were made with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on April 25, 2000.

Please amend the specification at page 84, line 27 to page 85, line 9 to read as follows:

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Using a proteomic-based approach, a novel protein was isolated from conditioned media obtained from squamous cell and colorectal carcinoma cell lines. The approach utilized in isolating this protein suggests that it is a naturally secreted product. The amino acid sequence of the isolated protein was determined and found to share sequence identity with EST sequences present in both GenBank® and proprietary (Amgen dbEST) databases. Specifically, the amino acid sequence of this novel secreted protein was found to share homology with an amino acid sequence corresponding to a human EST sequence present in the GenBank® database (~~GenBank~~ accession no. AA283751). The cDNA clone corresponding to this EST sequence was obtained from the Integrated Molecular Analysis of Genomes and their Expression (I.M.A.G.E.) Consortium (I.M.A.G.E. Consortium clone no. 713624). The nucleotide sequence of this cDNA clone was determined and was found to contain the complete coding sequence for the human Secs-1 gene.

Please amend the specification at page 85, lines 18-30 to read as follows:

Sequences corresponding to the murine Secs-1 gene were identified in proprietary databases derived from AC-6 stromal and colon crypt cDNA libraries. Murine Secs-1 polypeptide cDNA sequences were isolated as follows. Total RNA was prepared from either the AC-6 stromal cell line or colon crypt tissue using the TRIzol® method (~~Gibco~~ GIBCO-BRL, Rockville, MD), mRNA isolated from total RNA using a Dynabeads® mRNA kit (Dynal, Inc., Lake Success, NY), and cDNA synthesized from the isolated mRNA using the SuperScript[[™]]® System (~~Gibco~~ GIBCO-BRL) and a Not I oligo d(T) primer (~~Gibco~~ GIBCO-BRL). Following cDNA synthesis, Sal I adapters (~~Gibco~~ GIBCO-BRL) were ligated onto the cDNA, the cDNA was digested with Not I, and the digested cDNA was then size selected on an agarose gel. Inserts of approximately 1.0 kb or more in size were isolated and ligated into the pSPORT (~~Gibco~~ GIBCO-BRL) vector. Ligation products were

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transformed into DH10B competent cells and the nucleotide sequence of selected clones was then analyzed.

Please amend the specification at page 86, lines 8-23 to read as follows:

In addition to identifying a number of homologous sequences in GenBank® and proprietary databases, the human and murine Secs-1 nucleic acid sequences were found to share sequence homology with a previously reported rat EST sequence (Hennigan *et al.*, 1994, *Ocogene* 9:3591-600). However, Hennigan *et al.* disclosed only a portion of the rat Secs-1 nucleic acid sequence and did not disclose the open reading frame for the rat Secs-1 ortholog. Figure 3 illustrates the amino acid sequence alignment of the full-length rat Secs-1 polypeptide, murine Secs-1 polypeptide, and human Secs-1 polypeptide. A FASTA search of the ~~SwissProt~~ SWISS-PROT database using the predicted amino acid sequences for both human and murine Secs-1 polypeptide failed to identify any other sequences sharing significant homology.

Figures 4A-4F illustrate the genomic nucleotide sequence for human Secs-1 polypeptide. The genomic sequence for human Secs-1 polypeptide was determined by analysis of a publicly available BAC clone (GenBank® accession no. AC022389). The location and deduced amino acid sequence of the exons are indicated (Figures 4A, 4D, and 4F).

Please amend the specification at page 86, line 26 to page 87, line 12 to read as follows:

Multiple human tissue northern blots (either proprietary or obtained from BioSource Technologies, Inc., Hayward, CA or Clontech Laboratories, Inc., Palo Alto, CA) were probed with a ^{32}P-dCTP labeled, 542 bp human Secs-1 PCR fragment. The human Secs-1 PCR probe was prepared using 25 ng of Jurkat cell cDNA, amplimers corresponding to the human Secs-1 gene (5'-C-C-C-A-A-C-T-C-A-A-C-A-A-A-C-C-T-G-A-A-A-3'; SEQ ID NO: 14 and 5'-G-G-G-A-C-C-A-

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C-T-G-G-A-T-G-C-T-G-3'; SEQ ID NO: 15) at a final concentration of 0.2 μM each, 2.5 units of Taq polymerase, 200 μM dNTPs, 50 μCi ^{32}P-dCTP, and 1X Taq polymerase buffer, in a total reaction volume of 25 μl. Reactions were performed at 94°C for 2 minutes for one cycle; 94°C for 20 seconds, 57.8°C for 30 seconds, and 72°C for 1 minute for 40 cycles; and 72°C for 5 minutes for 1 cycle.

Northern blots were prehybridized for 1 hour at 42°C in ULTRAhyb[[™]]® (Ambion, Inc., Austin, TX), and then were hybridized at 42°C overnight in fresh hybridization buffer containing approximately 8 x 10^4 cpm/μl of the labeled probe. Following hybridization, the filters were washed twice for 30 minutes at 50°C in 2X SSPE and 0.5% SDS, and then twice for 15 minutes at room temperature in 0.1X SSPE and 0.5% SDS. The blots were then analyzed using a phosphoimager.

Please amend the specification at page 87, line 29 to page 88, line 25 to read as follows:

The riboprobe was obtained by *in vitro* transcription of a clone containing murine Secs-1 cDNA sequences. A murine Secs-1 cDNA sequence was generated by RT-PCR using 100 μg of total RNA from MSC 2.2.2 cells, amplimers corresponding to the murine Secs-1 gene (5'-A-C-T-C-C-G-G-C-T-C-C-T-T-C-A-C-T-A-T-G-A-3'; SEQ ID NO: 16 and 5'-A-T-G-T-G-G-G-C-A-T-C-A-T-C-A-A-C-G-C-T-T-T-A-3'; SEQ ID NO: 17) at a final concentration of 0.2 μM each, 5 units of Taq polymerase, 200 μM dNTPs, and 1X Taq polymerase buffer, in a total reaction volume of 50 μl. Reactions were performed at 94°C for 2 minutes for one cycle; 94°C for 20 seconds and 57°C for 30 seconds for 40 cycles; and 72°C for 5 minutes for 1 cycle. Following amplification, PCR products were separated on an agarose gel, excised from the gel, purified using the QIAQuick® Gel Extraction kit (~~Qiagen~~ QIAGEN Inc., Valencia, CA), and ligated into the pGEM-T (Promega Corp., Madison, WI) vector. Ligation products were transformed into DH5-α competent cells and six clones selected for analysis by restriction enzyme digestion and sequencing.

Following hybridization, sections were rinsed in buffer, treated with RNaseA to digest unhybridized probe, and then washed in 0.1X SSC at 55°C for 30 minutes. Sections were then immersed in NTB-2 emulsion (Eastman Kodak Co., Rochester, NY), exposed for 3 weeks at 4°C,

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developed, and counterstained with hematoxylin and eosin. Tissue morphology and hybridization signal were simultaneously analyzed by darkfield and standard illumination for brain (one sagittal and two coronal sections), gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, proximal colon, and distal colon), pituitary, liver, lung, heart, spleen, thymus, lymph nodes, kidney, adrenal, bladder, pancreas, salivary gland, male and female reproductive organs (ovary, oviduct, and uterus in the female; and testis, epididymus, prostate, seminal vesicle, and vas deferens in the male), BAT and WAT (subcutaneous, peri-renal), bone (femur), skin, breast, and skeletal muscle.

Please amend the specification at page 89, line 11 to page 90, line 2 to read as follows:

To prepare a human Secs-1 bacterial expression vector, a human Secs-1 cDNA sequence was first prepared by PCR amplification of 1 μg of a human Secs-1 cDNA clone in a reaction mix containing 0.4 *p*m/μl of amplimers corresponding to the human Secs-1 gene (5'-A-A-A-T-A-A-C-A-T-A-T-G-A-A-A-C-G-T-C-G-T-C-C-A-G-C-T-A-A-A-G-C-C-T-G-G-T-C-A-G-G-C-3'; SEQ ID NO: 18 and 5'-G-G-T-G-A-T-G-G-T-G-A-T-G-G-T-G-C-A-C-C-T-G-T-G-G-G-A-G-T-G-C-C-C-C-3'; SEQ ID NO: 19) and two Ready-To-Go® PCR Beads (Amersham Pharmacia Biotech AB Corp., Piscataway, NJ), in a total reaction volume of 50 μl. Reactions were performed at 94°C for 2 minutes for one cycle; 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 45 seconds for 30 cycles. The PCR product generated in this reaction was gel-purified on a 2% agarose gel.

The human Secs-1 PCR product produced in the initial amplification reaction was then re-amplified in a reaction mix containing 0.4 *p*m/μl of amplimers corresponding to the human Secs-1 gene (5'-A-A-A-T-A-A-C-A-T-A-T-G-A-A-A-C-G-T-C-G-T-C-C-A-G-C-T-A-A-A-G-C-C-T-G-G-T-C-A-G-G-C-3'; SEQ ID NO: 18 and 5'-G-T-G-G-T-A-G-T-G-G-T-A-G-T-G-G-T-A-G-T-A-A-C-T-A-T-C-C-T-A-G-G-T-A-T-T-T-3'; SEQ ID NO: 20) and two Ready-To-Go® PCR Beads, in a total reaction volume of 50 μl. Reactions were performed at 94°C for 2 minutes for one cycle; 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 45 seconds for 30 cycles. The ampliers used in the re-amplification reaction were designed to incorporate suitable restriction sites into the human Secs-1 cDNA sequence.

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Please amend the specification at page 92, lines 7-21 to read as follows:

At 8 weeks of age, transgenic founder animals and control animals are sacrificed for necropsy and pathological analysis. Portions of spleen are removed and total cellular RNA isolated from the spleens using the Total RNA Extraction Kit (~~Qiagen~~ QIAGEN Inc.) and transgene expression determined by RT-PCR. RNA recovered from spleens is converted to cDNA using the SuperScript[[™]]® Preamplification System (~~Gibco~~ GIBCO-BRL) as follows. A suitable primer, located in the expression vector sequence and 3' to the Secs-1 polypeptide transgene, is used to prime cDNA synthesis from the transgene transcripts. Ten mg of total spleen RNA from transgenic founders and controls is incubated with 1 mM of primer for 10 minutes at 70°C and placed on ice. The reaction is then supplemented with 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM of each dNTP, 0.1 mM DTT, and 200 U of SuperScript[[™]]® II reverse transcriptase. Following incubation for 50 minutes at 42°C, the reaction is stopped by heating for 15 minutes at 72°C and digested with 2U of RNase H for 20 minutes at 37°C. Samples are then amplified by PCR using primers specific for murine Secs-1 polypeptide.

Please amend the specification at page 93, lines 6-27 to read as follows:

The spleen, lymph node, and Peyer's patches of both the transgenic and the control mice are subjected to immunohistology analysis with B cell and T cell specific antibodies as follows. The formalin fixed paraffin embedded sections are deparaffinized and hydrated in deionized water. The sections are quenched with 3% hydrogen peroxide, blocked with Protein Block (Lipshaw Co., Pittsburgh, PA), and incubated in rat monoclonal anti-mouse B220 and CD3 (Harlan Bioproducts, Indianapolis, IN). Antibody binding is detected by biotinylated rabbit anti-rat immunoglobulins and peroxidase conjugated streptavidin (BioGenex Laboratories Corp., San Ramon, CA) with DAB as a chromagen (BioTek Solutions, Inc., Santa Barbara, CA). Sections are counterstained with hematoxylin.

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After necropsy, MLN and sections of spleen and thymus from transgenic animals and control littermates are removed. Single cell suspensions are prepared by gently grinding the tissues with the flat end of a syringe against the bottom of a 100 mm nylon cell strainer (Becton, Dickinson and Co., Franklin Lakes, NJ). Cells are washed twice, counted, and approximately 1 x 10^6 cells from each tissue are then incubated for 10 minutes with 0.5 μg CD16/32(FcγIII/II) Fc block in a 20 μL volume. Samples are then stained for 30 minutes at 2-8°C in a 100 μL volume of PBS (lacking Ca^+ and Mg^+), 0.1% bovine serum albumin, and 0.01% sodium azide with 0.5 μg antibody of FITC or PE-conjugated monoclonal antibodies against CD90.2 (Thy-1.2), CD45R (B220), CD11b(Mac-1), Gr-1, CD4, or CD8 (PharMingen Corp., San Diego, CA). Following antibody binding, the cells are washed and then analyzed by flow cytometry on a FACScan (Becton, Dickinson and Co.).

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